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## Protocol Article

# A practical synthesis of a novel DPAGT1 inhibitor, aminouridyl phenoxy piperidin benzyl butanamide (APPB) for in vivo studies



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## A B S T R A C T

Immunotherapy that targets *N*-linked glycans has not yet been developed due in large part to the lack of specificity of *N*-linked glycans between normal and malignant cells. *N*-Glycan chains are synthesized by the sequential action of glycosyl transferases in the Golgi apparatus. It is an overwhelming task to discover drug-like inhibitors of glycosyl transferases that block the synthesis of specific branching processes in cancer cells, killing tumor cells selectively. It has long been known that *N*-glycan biosynthesis can be inhibited by disruption of the first committed enzyme, dolichyl-phosphate *N*-acetylglucosaminephosphotransferase 1 (DPAGT1). Selective DPAGT1 inhibitors have the promising therapeutic potential for certain solid cancers that require increased branching of *N*-linked glycans in their growth progressions. Recently, we discovered that an anti-*Clostridium difficile* molecule, aminouridyl phenoxy piperidin benzyl butanamide (APPB) showed DPAGT1 inhibitory activity with the IC<sub>50</sub> value of 0.25 μM. It was confirmed that APPB inhibits *N*-glycosylation of β-catenin at 2.5 nM concentration. A sharp difference between APPB and tunicamycin was that the hemolytic activity of APPB is significantly attenuated (IC<sub>50</sub> > 200 μM RBC). Water solubility of APPB is >350-times greater than that of tunicamycin (78.8 mg/mL for APPB, <0.2 mg/mL for tunicamycin). A novel DPAGT1 inhibitor, APPB selectively inhibits growth of the solid tumors (e.g. KB, LoVo, SK-OV-3, MDA-MB-432S, HCT116, Panc-1, and AsPC-1) at low μM concentrations, but does not inhibit growth of a leukemia cell (L1210) and the healthy cells (Vero and HPNE) at these concentrations. *In vitro* metabolic stability using rat liver microsomes indicated that a half-life (*t*<sub>1/2</sub>) of APPB is sufficiently long (>60 min) for *in vivo* studies (PK/PD, safety profiles, and *in vivo* efficacy) using animal models. We have refined all steps in the previously reported synthesis for APPB for larger-scale. This article summarizes protocols of gram-scale synthesis of APPB and its physicochemical data, and a convenient DPAGT1 assay.

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## ARTICLE INFO

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## Specifications Table

Subject Area:	Chemistry
More specific subject area:	Medicinal Chemistry
Protocol name:	A practical synthesis of a novel DPAGT1 Inhibitor, aminouridyl phenoxypiperidinbenzyl butanamide (APPB) for <i>in vivo</i> studies
Reagents/tools:	All were operated with standard tools available in general synthetic and biochemistry lab.
Experimental design:	All synthetic steps were demonstrated in gram-quantity. Selectivity of all asymmetric reactions is greater than 15:1 ratio.
Trial registration:	N/A
Ethics:	N/A

## Value of the Protocol

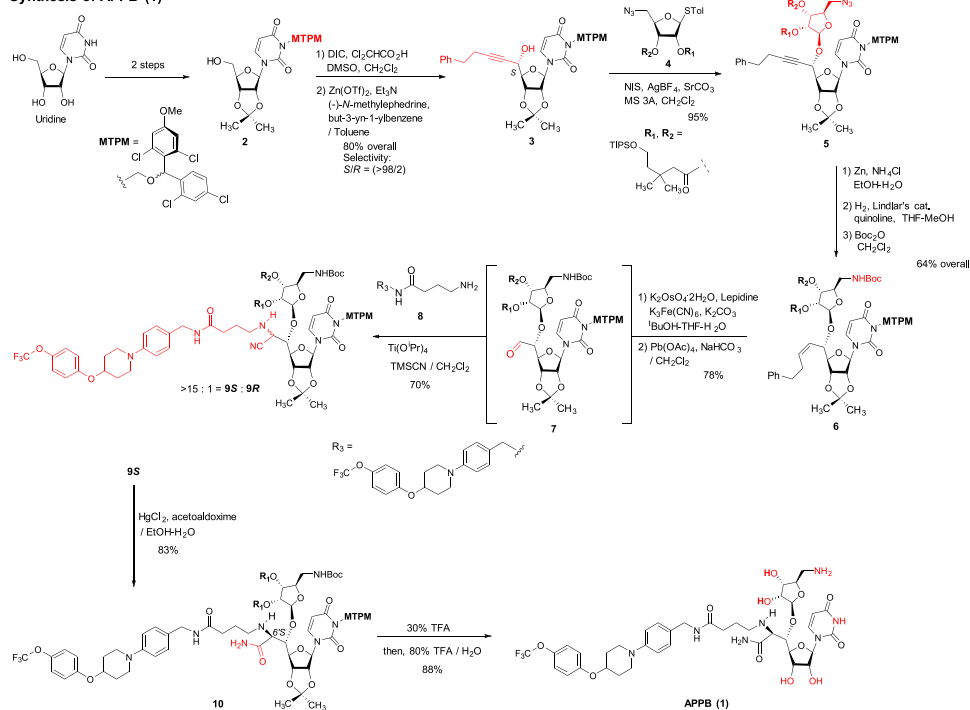
- All reactions were performed in over one gram-scale; the desired product was synthesized >1.0 g quantity.
- Synthesis of a novel DPAGT1 inhibitor
- Physicochemical property of a therapeutically interesting DPAGT1 inhibitor is summarized.

## Description of protocol

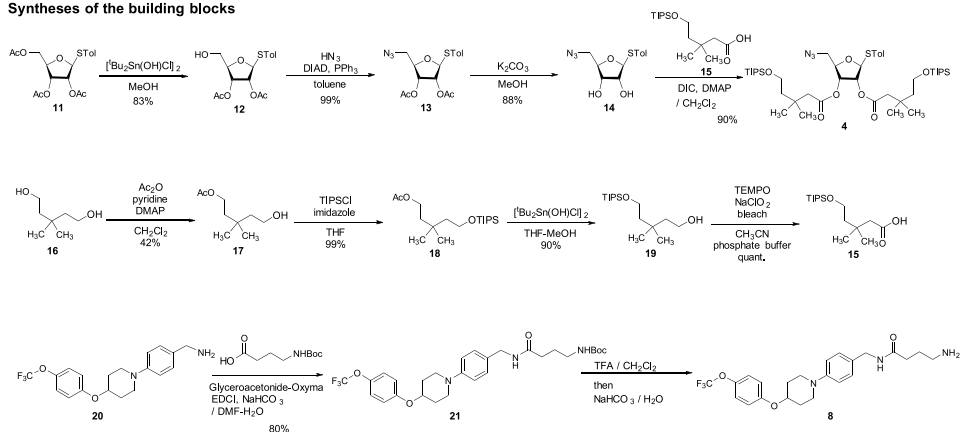
### Synthesis of a novel DPAGT1 inhibitor, aminouridyl phenoxypiperidinbenzyl butanamide (APPB, 1)

The monomethoxytetrachlorodiphenylmethoxymethyl (MTPM)-protected uridine **2** was prepared according to the previously reported procedure [1]. The *primary* alcohol of **2** was oxidized by a modified Swern condition to provide the corresponding aldehyde in quantitative yield, which was then subjected to Carreira's asymmetric alkylation reaction using (–)-*N*-methylephedrine [2], yielding the (*S*)-propargyl alcohol **3** in 80% yield with selectivity of >98:2. NIS-AgBF<sub>4</sub> promoted ribosylation of (*S*)-propargyl alcohol **3** with **4** furnished the β-riboside **5** exclusively in 95% yield. The azido group of **5** was reduced with Zn metal in the presence of aq. NH<sub>4</sub>Cl, and the triple bond was partially reduced with Lindlar's catalyst. The generated free-amine was protected with (Boc)<sub>2</sub>O to furnish **6** in 64% overall yield. The alkene moiety of **6** was subjected to a two-step procedure (osmylation and oxidative cleavage with Pb(OAc)<sub>4</sub>), providing the crude aldehyde **7**. Ti(O<sup>*i*</sup>Pr)<sub>4</sub>-mediated Strecker reaction of **7** with the 4-aminobutanamide derivatives **8** provided the *S*-diastereomer **9S** in 70% yield with greater than 15:1 *S/R* ratio. The desired diastereomer, **9S** was subjected to hydration reaction with HgCl<sub>2</sub>-acetoaldehyde, furnishing the amide **10** in 83% overall yield. Global deprotection of **10** was performed in one-pot two step reaction using 30% TFA in CH<sub>2</sub>Cl<sub>2</sub>

## Synthesis of APPB (1)



## Syntheses of the building blocks



Scheme 1. Synthesis of APPB (1).

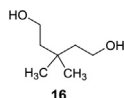
followed by 80% TFA in H<sub>2</sub>O; the crude product was purified by DOWEX 50W x 4 ion exchange resin followed by preparative HPLC to furnish **1** in 88% overall yield (Scheme 1).

## General

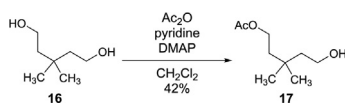
All chemicals were purchased from commercial sources and used without further purification unless otherwise noted. THF, CH<sub>2</sub>Cl<sub>2</sub>, and DMF were purified via Innovative Technology's Pure-Solve System. All reactions were performed under nitrogen atmosphere. Reactions were monitored by TLC

using 0.25 mm coated commercial silica gel plates (EMD, Silica Gel 60F<sub>254</sub>). TLC spots were visualized by UV light at 254 nm, or developed with ceric ammonium molybdate or anisaldehyde or copper sulfate or ninhydrin solutions by heating on a hot plate. Reactions were also monitored by using SHIMADZU LCMS-2020 with solvents: A: 0.1% formic acid in water, B: acetonitrile. Flash chromatography was performed with SiliCycle silica gel (Purasil 60 Å, 230–400 Mesh). <sup>1</sup>H NMR spectral data were recorded on 400, and 500 MHz instruments. <sup>13</sup>C NMR spectral data were recorded on 100 and 125 MHz instruments. For all NMR spectra, chemical shifts (δH, δC) were quoted in parts per million (ppm), and *J* values were quoted in Hz. <sup>1</sup>H and <sup>13</sup>C NMR spectra were calibrated with residual undeuterated solvent (CDCl<sub>3</sub>: δH = 7.26 ppm, δC = 77.16 ppm; CD<sub>3</sub>CN: δH = 1.94 ppm, δC = 1.32 ppm; CD<sub>3</sub>OD: δH = 3.31 ppm, δC = 49.00 ppm; DMSO-*d*<sub>6</sub>: δH = 2.50 ppm, δC = 39.52 ppm; D<sub>2</sub>O: δH = 4.79 ppm) as an internal reference. The following abbreviations were used to designate the multiplicities: s = singlet, d = doublet, dd = double doublets, t = triplet, q = quartet, quin = quintet, hept = heptet, m = multiplet, br = broad. Infrared (IR) spectra were recorded on a Perkin-Elmer FT1600 spectrometer. HPLC analyses were performed with a Shimadzu LC-20AD HPLC system. HR-MS data were obtained from a Waters Synapt G2-Si (ion mobility mass spectrometer with nano-electrospray ionization).

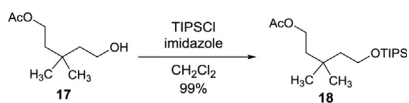
### Synthetic procedure for **1**



**3,3-Dimethylpentane-1,5-diol (16):** The title compound was synthesized according to the reported procedure [1,3]: TLC (hexanes/EtOAc 20:80) *R*<sub>f</sub> = 0.20; IR (thin film)  $\nu_{\text{max}}$  = 3317 (br), 2955, 2934, 1676, 1469, 1366, 1030, 1006, 990 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.73 (t, *J* = 7.0 Hz, 4H), 2.04 (brs, 2H), 1.57 (t, *J* = 7.0 Hz, 4H), 0.94 (s, 6H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 59.60 (2C), 44.06 (2C), 31.67, 28.08 (2C); HRMS (ESI+) *m/z* calcd for C<sub>7</sub>H<sub>16</sub>O<sub>2</sub> 132.1150, found 132.1144.

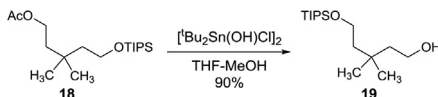


**5-Hydroxy-3,3-dimethylpentyl acetate (17):** To a stirred solution of **16** (47.5 g, 359.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (500 mL) were added pyridine (31.8 mL, 395.2 mmol), Ac<sub>2</sub>O (33.9 mL, 359.3 mmol) and DMAP (0.44 g, 3.59 mmol) at 0 °C. The reaction mixture was stirred for 12 h at rt, and all volatiles were evaporated *in vacuo*. Purification by silica gel column chromatography (hexanes/EtOAc 90:10 to 50:50) to gave **17** (26.3 g, 150.9 mmol, 42%): TLC (hexanes/EtOAc 67:33) *R*<sub>f</sub> = 0.20; <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 4.13 (t, *J* = 7.5 Hz, 2H), 3.72 (t, *J* = 7.5 Hz, 2H), 2.04 (s, 3H), 1.57 (dt, *J* = 14.8, 7.5 Hz, 4H), 0.95 (s, 6H); HRMS (ESI+) *m/z* calcd for C<sub>9</sub>H<sub>18</sub>O<sub>3</sub> 174.1256, found 174.1249.

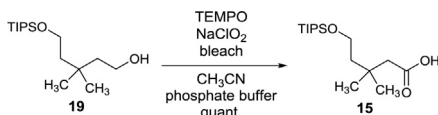


**3,3-Dimethyl-5-((triisopropylsilyl)oxy)pentyl acetate (18):** To a stirred solution of **17** (26.3 g, 150.9 mmol) and imidazole (20.6 g, 301.8 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (500 mL) were added TIPSCl (48.4 mL, 226.4 mmol) and DMAP (0.18 g, 1.51 mmol) at 0 °C. The reaction mixture was warmed to rt and stirred for 16 h. The reaction was quenched with saturated NaHCO<sub>3</sub> (aq.) and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 97:3) to obtain **18** (49.4 g, 149.4 mmol, 99%): TLC (hexanes/EtOAc 90:10) *R*<sub>f</sub> = 0.70; <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 4.12 (t, *J* = 7.6 Hz, 2H),

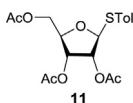
3.74 (t,  $J = 7.2$  Hz, 2H), 2.03 (s, 3H), 1.59 (t,  $J = 7.6$  Hz, 2H), 1.53 (t,  $J = 7.2$  Hz, 2H), 1.11–1.02 (m, 21H), 0.94 (s, 6H); HRMS (ESI+)  $m/z$  calcd for  $C_{18}H_{39}O_3Si$   $[M+H]^+$  331.2668, found 331.2685.



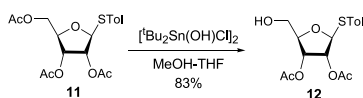
**3,3-Dimethyl-5-((triisopropylsilyl)oxy)pentan-1-ol (19):** To a stirred solution of **18** (49.4 g, 149.4 mmol) in MeOH/THF (4:1, 300 mL) was added  $[tBu_2Sn(OH)Cl]_2$  (0.86 g, 1.50 mmol). After 20 h at rt, all volatiles were evaporated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 95:5 to 90:10) to provide **19** (38.8 g, 134.5 mmol, 90%); TLC (hexanes/EtOAc 80:20)  $R_f = 0.40$ ; IR (thin film)  $\nu_{max} = 3343$  (br), 2941, 2891, 2866, 1463, 1384, 1366, 1096, 1065, 1012, 995, 881, 745, 678, 656  $cm^{-1}$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  3.76 (t,  $J = 6.9$  Hz, 2H), 3.72 (t,  $J = 7.2$  Hz, 2H), 1.57 (td,  $J = 7.1, 2.8$  Hz, 4H), 1.12–1.03 (m, 21H), 0.94 (s, 6H);  $^{13}C$  NMR (101 MHz,  $CDCl_3$ )  $\delta$  60.30, 59.85, 44.31, 31.67, 28.14 (2C), 18.05 (6C), 11.95 (3C); HRMS (ESI+)  $m/z$  calcd for  $C_{16}H_{36}O_2Si$  288.2485, found 288.2473.



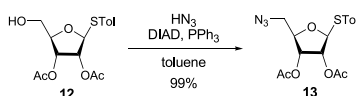
**3,3-Dimethyl-5-((triisopropylsilyl)oxy)pentanoic acid (15):** To a stirred solution of **19** (38.8 g, 134.5 mmol) and TEMPO (1.05 g, 6.73 mmol) in MeCN (135 mL) and a phosphate buffer (pH = 6.8, 135 mL) were added  $NaClO_2$  (14.6 g, 141.4 mmol) and bleach (8.25%, 65 mL) at 35 °C. After being stirred for 4 h, the reaction mixture was extracted with EtOAc and combined organic phase was dried over  $Na_2SO_4$  and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 90:10) to give **15** (40.7 g, 134.5 mmol, 100%) as an orange oil; TLC (hexanes/EtOAc 50:50)  $R_f = 0.50$ ; IR (thin film)  $\nu_{max} = 2942, 2866, 1705, 1463, 1246, 1097, 996, 881, 738, 678$   $cm^{-1}$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  3.88 (t,  $J = 5.8$  Hz, 2H), 2.38 (s, 2H), 1.71 (t,  $J = 5.8$  Hz, 2H), 1.20–1.11 (m, 3H), 1.09 (s, 12H), 1.08 (s, 6H), 1.07 (s, 6H);  $^{13}C$  NMR (101 MHz,  $CDCl_3$ )  $\delta$  173.9, 60.7, 46.8, 42.6, 32.4, 28.5 (2C), 17.9 (6C), 11.8 (3C); HRMS (ESI+)  $m/z$  calcd for  $C_{16}H_{34}O_3NaSi$   $[M+Na]^+$  325.2175, found 325.2171.



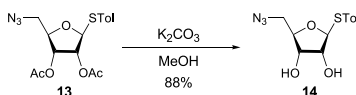
**(2R,3S,4S,5S)-2-(Acetoxymethyl)-5-(p-tolylthio)tetrahydrofuran-3,4-diyl diacetate (10):** The title compound was synthesized according to the reported procedure [1]; TLC (hexanes/EtOAc 50:50)  $R_f = 0.60$ ;  $[\alpha]^{20}_D -0.411$  ( $c = 0.51$ ,  $CHCl_3$ ); IR (thin film)  $\nu_{max} = 1742, 1371, 1214, 1091, 1045, 1017, 899, 810$   $cm^{-1}$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.41 (d,  $J = 8.1$  Hz, 2H), 7.14 (d,  $J = 7.7$  Hz, 2H), 5.25–5.22 (m, 1H), 5.21–5.17 (m, 2H), 4.26–4.20 (m, 2H), 4.07 (dd,  $J = 12.9, 5.5$  Hz, 1H), 2.34 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H);  $^{13}C$  NMR (101 MHz,  $CDCl_3$ )  $\delta$  170.50, 169.63, 169.42, 138.80, 134.18 (2C), 129.81 (2C), 127.45, 87.95, 79.97, 73.67, 71.41, 63.46, 21.15, 20.75, 20.53 (2C); HRMS (ESI+)  $m/z$  calcd for  $C_{18}H_{22}O_7NaS$   $[M+Na]^+$  405.0984, found: 405.0970.



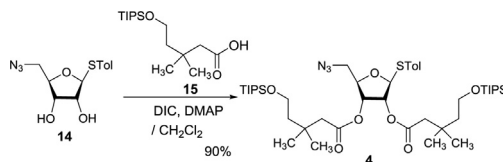
**(2R,3S,4S,5S)-2-(Hydroxymethyl)-5-(p-tolylthio)tetrahydrofuran-3,4-diyl diacetate (12):** To a stirred solution of **10** (24.3 g, 62.8 mmol) in MeOH/THF (4:1, 300 mL) was added [ $^t\text{Bu}_2\text{Sn}(\text{OH})\text{Cl}$ ] $_2$  (0.72 g, 1.26 mmol). After 20 h at rt, all volatiles were evaporated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 67:33) to provide **11** (17.9 g, 52.7 mmol, 83%); TLC (hexanes/EtOAc 60:40)  $R_f$ =0.40;  $[\alpha]^{21}_D$  −0.298 ( $c$  = 1.37,  $\text{CHCl}_3$ ); IR (thin film)  $\nu_{\text{max}}$  = 3484 (br), 3021, 2924, 2877, 1746, 1493, 1432, 1373, 1239, 1222, 1102, 1093, 1046, 1017, 810  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.43 (d,  $J$  = 7.8 Hz, 2H), 7.16 (d,  $J$  = 7.8 Hz, 2H), 5.27 (d,  $J$  = 5.6 Hz, 1H), 5.24 (t,  $J$  = 4.6 Hz, 1H), 5.20 (d,  $J$  = 5.8 Hz, 1H), 4.13 (q,  $J$  = 3.7 Hz, 1H), 3.74 (dd,  $J$  = 12.3, 2.8 Hz, 1H), 3.58 (dd,  $J$  = 12.2, 3.2 Hz, 1H), 2.34 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  169.89, 169.39, 138.92, 133.88 (2C), 129.93 (2C), 127.45, 87.76, 83.46, 73.89, 71.40, 62.08, 21.17, 20.62, 20.57; HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{16}\text{H}_{21}\text{O}_6\text{S}$  [ $\text{M}+\text{H}$ ] 341.1059, found 341.1075.



**(2R,3S,4S,5S)-2-(Azidomethyl)-5-(p-tolylthio)tetrahydrofuran-3,4-diyl diacetate (13):** To a stirred solution of **12** (17.9 g, 52.7 mmol) and  $\text{PPh}_3$  (27.6 g, 105.1 mmol) in dry toluene (100 mL) were added  $\text{HN}_3$  (1.0 M in toluene, 262.9 mL, 262.9 mmol) and DIAD (20.7 mL, 105.1 mmol). The reaction mixture was stirred for 8 h at rt, and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 80:20 to 70:30) to afford **13** (19.0 g, 52.0 mmol, 99%); TLC (hexanes/EtOAc 75:25)  $R_f$ =0.40;  $[\alpha]^{21}_D$  −0.899 ( $c$  = 3.93,  $\text{CHCl}_3$ ); IR (thin film)  $\nu_{\text{max}}$  = 3023, 2924, 2101, 1746, 1493, 1436, 1372, 1233, 1217, 1094, 1064, 1044, 1016, 965, 899, 810  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.43 (d,  $J$  = 8.1 Hz, 2H), 7.15 (d,  $J$  = 7.9 Hz, 2H), 5.27 (d,  $J$  = 5.2 Hz, 1H), 5.19 (t,  $J$  = 5.3 Hz, 1H), 5.11 (t,  $J$  = 5.2 Hz, 1H), 4.15 (q,  $J$  = 5.0 Hz, 1H), 3.42 (d,  $J$  = 1.2 Hz, 1H), 3.41 (d,  $J$  = 2.2 Hz, 1H), 2.34 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  169.63, 169.35, 138.74, 133.86 (2C), 129.81 (2C), 127.60, 88.27, 80.97, 73.74, 71.73, 52.46, 21.14, 20.50, 20.49; HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{16}\text{H}_{20}\text{N}_3\text{O}_5\text{S}$  [ $\text{M}+\text{H}$ ] 366.1124, found: 366.1133.

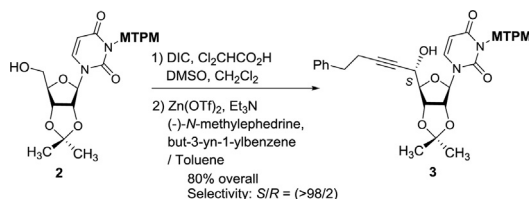


**(2R,3R,4S,5S)-2-(Azidomethyl)-5-(p-tolylthio)tetrahydrofuran-3,4-diol (13):** To a stirred solution of **13** (19.0 g, 52.0 mmol) in MeOH (200 mL) was added  $\text{K}_2\text{CO}_3$  (10.0 g, 72.5 mmol). After being stirred for 30 min, the reaction mixture was filtered and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 70:30 to 50:50) to afford **14** (12.9 g, 45.9 mmol, 88%); TLC (hexanes/EtOAc 33:67)  $R_f$ =0.60;  $[\alpha]^{21}_D$  −0.152 ( $c$  = 0.34,  $\text{CHCl}_3$ ); IR (thin film)  $\nu_{\text{max}}$  = 3385 (br), 2923, 2103, 1493, 1437, 1399, 1286, 1117, 1065, 1042, 1017, 809  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.42 (d,  $J$  = 7.7 Hz, 2H), 7.14 (d,  $J$  = 7.8 Hz, 2H), 5.19 (d,  $J$  = 4.7 Hz, 1H), 4.11 (t,  $J$  = 4.4 Hz, 1H), 4.04 (d,  $J$  = 3.7 Hz, 2H), 3.49 (dd,  $J$  = 13.0, 2.9 Hz, 1H), 3.42 (dd,  $J$  = 13.0, 4.2 Hz, 1H), 2.57 (brs, 2H), 2.34 (s, 3H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  138.34, 133.17 (2C), 129.82 (2C), 128.68, 90.76, 82.62, 74.88, 72.24, 52.68, 21.15; HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{12}\text{H}_{16}\text{N}_3\text{O}_3\text{S}$  [ $\text{M}+\text{H}$ ] 282.0912, found: 282.0928.

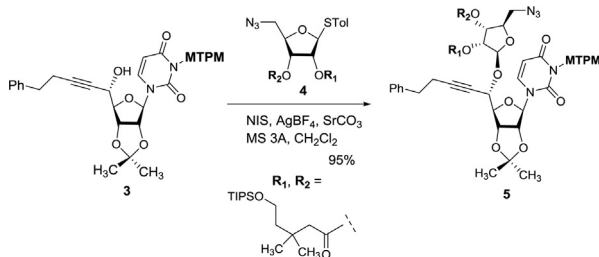


**(2R,5S)-2-(Azidomethyl)-5-(p-tolylthio)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyl)oxy)pentanoate) (4):** To a stirred solution of **14** (12.9 g, 45.9 mmol) and **15** (34.7 g, 114.8 mmol) in  $\text{CH}_2\text{Cl}_2$  (231 mL) were added DMAP (1.12 g, 9.17 mmol) and DIC (18.0 mL, 114.8 mmol) at 0 °C. The reaction mixture was stirred for 16 h at rt and concentrated *in vacuo*. The crude mixture

was purified by silica gel column chromatography (hexanes/EtOAc 95:5) to afford **4** (35.1 g, 41.2 mmol, 90%): TLC (hexanes/EtOAc 90:10)  $R_f$  = 0.60;  $[\alpha]_D^{21}$   $-0.293$  ( $c$  = 1.39,  $\text{CHCl}_3$ ); IR (thin film)  $\nu_{\text{max}}$  = 2792, 2892, 2866, 2102, 1745, 1464, 1390, 1367, 1282, 1254, 1219, 1190, 1100, 1071, 1054, 1013, 998, 882, 809, 772, 742, 681  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.43 (d,  $J$  = 8.1 Hz, 2H), 7.14 (d,  $J$  = 7.9 Hz, 2H), 5.26 (d,  $J$  = 5.3 Hz, 1H), 5.18 (t,  $J$  = 5.3 Hz, 1H), 5.11 (t,  $J$  = 5.0 Hz, 1H), 4.13 (q,  $J$  = 4.7 Hz, 1H), 3.76 (dt,  $J$  = 10.6, 6.9 Hz, 4H), 3.42 (d,  $J$  = 4.7 Hz, 2H), 2.34 (s, 3H), 2.31 (d,  $J$  = 10.6 Hz, 2H), 2.26 (d,  $J$  = 4.9 Hz, 2H), 1.61 (dtd,  $J$  = 17.4, 6.9, 2.1 Hz, 4H), 1.08–1.00 (m, 54H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  170.91, 170.54, 138.67, 133.89 (2C), 129.81 (2C), 127.88, 88.58, 81.48, 73.52, 71.70, 60.02, 59.97, 52.70, 46.15, 46.03, 44.64, 44.55, 32.68, 32.60, 27.51, 27.47, 27.38, 21.17, 18.06 (6C), 18.05 (6C), 11.93 (3C), 11.92 (3C); HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{44}\text{H}_{79}\text{N}_3\text{NaO}_7\text{SSi}_2$  [ $\text{M}+\text{Na}$ ] 872.5075, found: 872.5088.

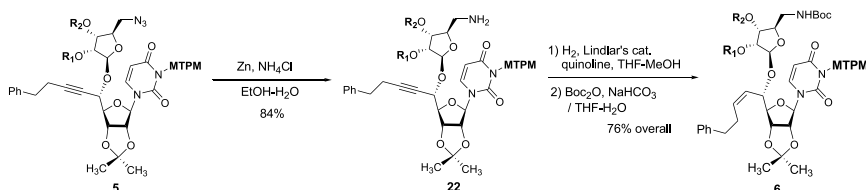


**3-(((2,6-Dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-1-((3aR,4R,6R,6aR)-6-((S)-1-hydroxy-5-phenylpent-2-yn-1-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)pyrimidine-2,4-(1H,3H)-dione (3):** Title compound was synthesized according to the reported procedure [1]: TLC (hexanes/EtOAc 50:50)  $R_f$  = 0.30;  $[\alpha]_D^{22}$   $-0.116$  ( $c$  = 2.17,  $\text{CHCl}_3$ ); IR (thin film)  $\nu_{\text{max}}$  = 3387 (br), 2981, 2937, 1664, 1454, 1276, 1065, 1039, 856, 733, 698  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.53 (ddd,  $J$  = 20.4, 8.5, 0.7 Hz, 1H), 7.35–7.27 (m, 4H), 7.24–7.15 (m, 4H), 6.85 (d,  $J$  = 5.1 Hz, 2H), 6.51 (d,  $J$  = 5.4 Hz, 1H), 5.68 (dd,  $J$  = 8.1, 4.1 Hz, 1H), 5.60–5.50 (m, 3H), 4.89–4.78 (m, 2H), 4.57 (ddt,  $J$  = 12.0, 4.3, 2.0 Hz, 1H), 4.24 (dd,  $J$  = 4.4, 3.1 Hz, 1H), 3.78 (d,  $J$  = 3.3 Hz, 3H), 2.83 (t,  $J$  = 7.5 Hz, 2H), 2.53 (td,  $J$  = 7.4, 2.0 Hz, 2H), 1.57 (s, 3H), 1.36 (s, 3H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  162.11, 162.08, 159.5, 150.87, 150.85, 141.1, 140.8, 140.30, 140.27, 136.9, 135.4, 135.3, 133.99, 133.95, 133.8, 133.6, 131.2, 129.4, 129.3, 128.41, 128.39, 126.4, 126.21, 126.18, 125.5, 125.4, 115.34, 115.32, 114.3, 114.2, 101.8, 101.7, 96.7, 96.4, 89.23, 89.19, 86.8, 86.7, 84.1, 84.0, 80.9, 69.5, 63.02, 62.99, 55.7, 34.72, 34.70, 27.2, 25.3, 20.87, 20.85; HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{37}\text{H}_{34}\text{N}_2\text{O}_8\text{NaCl}_4$  [ $\text{M}+\text{Na}$ ] 797.0967, found: 797.0994.



**(2R,3R,4R,5R)-2-(Azidomethyl)-5-(((1S)-1-((3aR,4R,6R,6aR)-6-3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-phenylpent-2-yn-1-yl)oxy)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-(((triisopropylsilyl)oxy)pentanoate) (5):** To a stirred suspension of **3** (5 g, 6.44 mmol), **4** (6.57 g, 7.73 mmol), MS3A (7.56 g) and  $\text{SrCO}_3$  (4.75 g, 32.2 mmol) in  $\text{CH}_2\text{Cl}_2$  (260 mL) were added  $\text{AgBF}_4$  (0.63 g, 3.22 mmol) and NIS (1.88 g, 8.37 mmol) at  $0^\circ\text{C}$ . After 24 h, the reaction mixture was added  $\text{Et}_3\text{N}$  (2 mL) and passed through a silica gel pad (hexanes/EtOAc 1:1). The combined organic phase was concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 90:10 to 80:20 to 70:30) to afford **5** (9.19 g, 6.12 mmol, 95%): TLC (hexanes/EtOAc 67:33)  $R_f$  = 0.70;  $[\alpha]_D^{21}$   $+0.100$  ( $c$  = 2.09,  $\text{CHCl}_3$ ); IR (thin film)  $\nu_{\text{max}}$  = 2942, 2866, 2102, 1743, 1724, 1675, 1456, 1278, 1218, 1099, 1070, 882, 772  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.54

(dd,  $J = 23.1, 8.5$  Hz, 1H), 7.32–7.27 (m, 4H), 7.24–7.16 (m, 4H), 6.84 (d,  $J = 7.3$  Hz, 2H), 6.51 (d,  $J = 3.7$  Hz, 1H), 5.71–5.64 (m, 2H), 5.60–5.49 (m, 2H), 5.20–5.16 (m, 3H), 4.79 (ddd,  $J = 7.5, 6.5, 3.1$  Hz, 1H), 4.64 (td,  $J = 5.9, 2.6$  Hz, 1H), 4.57 (ddt,  $J = 11.4, 6.3, 1.9$  Hz, 1H), 4.28 (dt,  $J = 6.2, 2.8$  Hz, 1H), 4.19 (tt,  $J = 6.1, 3.0$  Hz, 1H), 3.79–3.72 (m, 7H), 3.50 (ddd,  $J = 13.0, 7.6, 3.3$  Hz, 1H), 3.35 (dd,  $J = 13.0, 3.4$  Hz, 1H), 2.83 (t,  $J = 7.4$  Hz, 2H), 2.55 (td,  $J = 7.4, 1.8$  Hz, 2H), 2.29 (t,  $J = 1.6$  Hz, 2H), 2.24 (dd,  $J = 5.1, 2.1$  Hz, 2H), 1.62–1.55 (m, 7H), 1.36 (d,  $J = 2.0$  Hz, 3H), 1.08–1.00 (m, 54H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  175.6, 171.0, 170.9, 170.71, 170.70, 170.6, 162.2, 162.1, 159.5, 150.8, 150.7, 140.4, 140.19, 140.15, 140.13, 136.92, 136.91, 135.4, 135.3, 133.9, 133.8, 133.7, 131.2, 129.4, 129.3, 128.5 (2C), 128.4 (2C), 126.5, 126.4, 126.2, 126.1, 125.6, 125.5, 115.29, 115.25, 114.23, 114.22, 104.61, 104.55, 101.83, 101.82, 88.8, 88.2, 84.44, 84.35, 83.9, 81.4, 81.3, 80.6, 79.9, 76.5, 75.9, 75.8, 74.1, 71.8, 71.7, 71.4, 70.7, 69.6, 69.5, 68.9, 68.8, 59.97, 59.96, 55.7, 46.2, 46.0, 44.7, 44.6, 34.7, 34.51, 34.49, 32.7, 32.61, 32.57, 28.0, 27.38, 27.35, 27.3, 27.1, 25.34, 25.27, 20.9, 18.1 (12C), 11.9 (6C); HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{74}\text{H}_{106}\text{Cl}_4\text{N}_5\text{O}_{15}\text{Si}_2$  [ $\text{M}+\text{H}$ ] 1500.5978, found: 1500.5992.

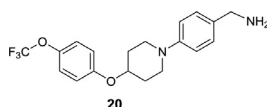


**(2R,3R,4R,5R)-2-((Aminomethyl)-5-(((1S)-1-((3aR,4R,6R,6aR)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-phenylpent-2-yn-1-yl)oxy)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyl)oxy)pentanoate) (22):** A suspended solution of **5** (7.03 g, 4.68 mmol),  $\text{NH}_4\text{Cl}$  (7.50 g, 140.3 mmol) and  $\text{Zn}$  (9.17 g, 140.3 mmol) in  $\text{EtOH}/\text{H}_2\text{O}$  (9:1, 50 mL) was stirred at  $80^\circ\text{C}$  for 12 h and cooled to rt. The precipitates were filtered and the combined organic solution was concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/ $\text{EtOAc}$  50:50 to  $\text{CHCl}_3/\text{MeOH}$  96:4) to afford the primary amine **22** (5.80 g, 3.93 mmol, 84%); TLC ( $\text{CHCl}_3/\text{MeOH}$  90:10)  $R_f = 0.60$ ;  $[\alpha]_D^{21} -0.013$  ( $c = 1.35$ ,  $\text{CHCl}_3$ ); IR (thin film)  $\nu_{\text{max}} = 2941, 2866, 1742, 1721, 1675, 1600, 1556, 1461, 1382, 1278, 1215, 1099, 1070, 1050, 999, 882\text{ cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.64 (s, 1H), 7.50 (dd,  $J = 31.4, 8.4$  Hz, 1H), 7.33–7.30 (m, 2H), 7.28 (d,  $J = 7.5$  Hz, 3H), 7.24–7.15 (m, 5H), 6.85 (d,  $J = 9.5$  Hz, 2H), 6.49 (d,  $J = 6.1$  Hz, 1H), 5.75 (dd,  $J = 8.5, 1.9$  Hz, 1H), 5.72–5.66 (m, 1H), 5.59–5.46 (m, 2H), 5.30 (d,  $J = 5.3$  Hz, 1H), 5.22–5.13 (m, 2H), 4.82 (dt,  $J = 6.3, 3.1$  Hz, 1H), 4.78 (d,  $J = 7.0$  Hz, 1H), 4.65 (dd,  $J = 14.5, 7.6$  Hz, 1H), 4.28 (dt,  $J = 7.4, 3.5$  Hz, 1H), 4.17 (quin,  $J = 3.9$  Hz, 1H), 3.87 (t,  $J = 5.8$  Hz, 1H), 3.75 (dt,  $J = 15.3, 6.3$  Hz, 6H), 3.14 (d,  $J = 13.6$  Hz, 1H), 2.94–2.86 (m, 1H), 2.83 (t,  $J = 7.4$  Hz, 2H), 2.55 (td,  $J = 7.2, 2.0$  Hz, 2H), 2.35 (s, 1H), 2.30 (s, 2H), 2.25 (s, 2H), 2.23–2.17 (m, 1H), 1.70 (t,  $J = 5.9$  Hz, 1H), 1.63–1.51 (m, 4H), 1.35 (d,  $J = 5.1$  Hz, 2H), 1.25 (s, 1H), 1.12–0.95 (m, 51H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  173.46, 171.73, 171.39, 170.66, 162.24, 159.45, 150.84, 140.16, 136.82, 135.21, 135.04, 134.04, 133.95, 133.75, 131.18, 131.16, 131.14, 129.40, 129.35, 128.52, 128.43 (2C), 128.40 (2C), 128.37, 126.42, 126.29, 126.16, 125.40, 125.26, 115.30, 115.24, 114.01, 101.82, 89.55, 84.49, 74.87, 70.13, 60.70, 59.93, 55.69, 47.00, 46.16, 45.95, 44.74, 44.64, 42.72, 34.53, 34.51, 32.62, 32.58, 32.33, 29.69, 28.47, 27.38, 27.34, 27.29, 27.03, 25.21, 25.19, 20.92, 18.04 (12C), 17.91, 11.87 (6C), 11.78; HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{74}\text{H}_{108}\text{Cl}_4\text{N}_5\text{O}_{15}\text{Si}_2$  [ $\text{M}+\text{H}$ ] 1474.6073, found: 1475.6791.

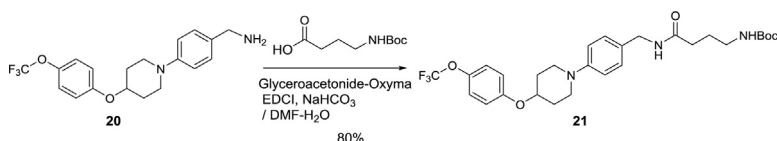
**(2R,3R,4R,5R)-2-(((tert-Butoxycarbonyl)amino)methyl)-5-(((1S,Z)-1-((3aR,4R,6R,6aR)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-phenylpent-2-en-1-yl)oxy)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyl)oxy)pentanoate) (6):** To a stirred solution of **22** (5.80 g, 3.93 mmol) and quinoline (10 mL) in  $\text{THF-MeOH}$  (1:1, 200 mL) was added Lindlar catalyst (2.90 g).  $\text{H}_2$  gas was introduced and the reaction mixture was stirred under  $\text{H}_2$  atmosphere (1000 psi). After being stirred for 20 h, the reaction mixture was added Lindlar catalyst (2.90 g). The reaction mixture was stirred for 20 h under  $\text{H}_2$  atmosphere (1000 psi) at rt. The solution was filtered through Celite, concentrated *in vacuo*. The crude mixture was used for the next reaction without purification. To a stirred solution of the crude mixture in  $\text{CH}_2\text{Cl}_2$  (40 mL) was  $\text{Boc}_2\text{O}$  (1.29 g,



5.89 mmol). After being stirred for 12 h at rt, the reaction mixture was quenched with 1N HCl and extracted with EtOAc. The combined organic solution was washed with saturated aq. NaHCO<sub>3</sub>, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude mixture was passed through a silica gel pad (hexanes/EtOAc 80:20 to 70:30), to afford **6** (4.71 g, 2.99 mmol, 76%): TLC (hexanes/EtOAc 75:25)  $R_f$  = 0.40;  $[\alpha]_D^{21}$  –0.015 ( $c$  = 0.86, CHCl<sub>3</sub>); IR (thin film)  $\nu_{\max}$  = 3403 (br), 2957, 2941, 2866, 1720, 1675, 1600, 1556, 1507, 1456, 1382, 1367, 1278, 1247, 1218, 1161, 1100, 1071, 1049, 1013, 999, 882 cm<sup>–1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.54 (dd,  $J$  = 8.4, 6.7 Hz, 1H), 7.35 (d,  $J$  = 8.2 Hz, 1H), 7.30 (t,  $J$  = 2.1 Hz, 2H), 7.25–7.21 (m, 2H), 7.21–7.12 (m, 5H), 6.82 (d,  $J$  = 10.7 Hz, 2H), 6.51 (d,  $J$  = 13.6 Hz, 1H), 5.84–5.74 (m, 2H), 5.72 (d,  $J$  = 8.1 Hz, 1H), 5.62–5.50 (m, 2H), 5.47 (t,  $J$  = 8.0 Hz, 1H), 5.14 (t,  $J$  = 4.2 Hz, 1H), 5.07–4.97 (m, 2H), 4.90 (s, 1H), 4.75 (ddd,  $J$  = 24.4, 6.4, 2.0 Hz, 1H), 4.58–4.45 (m, 2H), 4.19 (dt,  $J$  = 8.4, 4.2 Hz, 1H), 4.01 (dt,  $J$  = 6.6, 4.3 Hz, 1H), 3.76 (d,  $J$  = 5.1 Hz, 4H), 3.75–3.70 (m, 4H), 3.32 (d,  $J$  = 5.0 Hz, 2H), 2.79–2.58 (m, 2H), 2.57–2.41 (m, 2H), 2.36–2.28 (m, 1H), 2.26–2.19 (m, 5H), 1.66–1.52 (m, 4H), 1.41 (s, 6H), 1.33 (d,  $J$  = 4.9 Hz, 2H), 1.05 (q,  $J$  = 2.7 Hz, 5H), 0.99 (dd,  $J$  = 9.6, 4.0 Hz, 4H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  170.87, 159.38, 155.94, 150.85, 141.06, 136.87, 136.80, 135.57, 135.31, 135.27, 133.86, 133.66, 133.56, 131.23, 131.20, 129.29, 129.27, 128.52, 128.51, 128.37 (2C), 126.16, 126.15, 126.06, 126.03, 125.99, 125.64, 125.52, 125.46, 125.43, 115.24, 115.23, 114.17, 114.11, 84.61, 81.15, 81.03, 79.30, 79.25, 74.72, 74.29, 70.50, 69.81, 59.95, 59.91, 55.66, 55.65, 46.18, 46.17, 45.92, 44.80, 44.79, 41.64, 35.37, 35.34, 32.56, 32.55, 32.52, 32.50, 29.70, 28.34, 27.27, 27.24, 27.22, 27.10, 27.08, 25.25, 18.05 (12C), 17.88, 11.88 (6C), 11.74; HRMS (ESI+)  $m/z$  calcd for C<sub>79</sub>H<sub>118</sub>Cl<sub>4</sub>N<sub>3</sub>O<sub>17</sub>Si<sub>2</sub> [M+H] 1576.6754, found: 1576.6771.

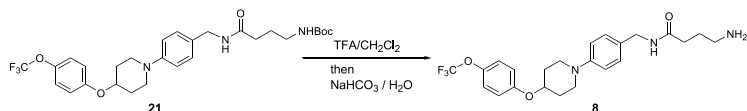


**(4-(4-(4-(Trifluoromethoxy)phenoxy)piperidin-1-yl)phenyl)methanamine (20):** The title compound was synthesized according to the reported procedure [5]: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.21 (d,  $J$  = 8.2 Hz, 2H), 7.14 (d,  $J$  = 8.6 Hz, 2H), 6.97–6.87 (m, 4H), 4.43 (tt,  $J$  = 7.7, 3.8 Hz, 1H), 3.79 (s, 2H), 3.49 (ddd,  $J$  = 11.7, 7.2, 3.7 Hz, 2H), 3.09 (ddd,  $J$  = 12.2, 8.2, 3.6 Hz, 2H), 2.15–2.06 (m, 2H), 1.98–1.88 (m, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  155.8, 150.2, 142.8, 134.6, 128.0 (2C), 122.5 (2C), 116.83 (2C), 116.76 (2C), 72.9, 46.9 (2C), 45.9, 30.4 (2C); HRMS (ESI+)  $m/z$  calcd for C<sub>19</sub>H<sub>22</sub>F<sub>3</sub>N<sub>2</sub>O<sub>2</sub> [M+H] 367.1633, found 367.1628.

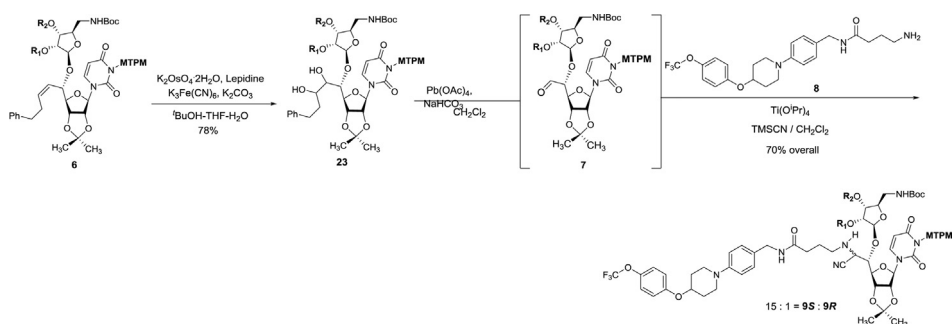


**tert-Butyl (4-oxo-4-((4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)amino)butyl)carbamate (21):** To a stirred solution of 4-aminobutyric acid (2.50 g, 24.0 mmol) and NaHCO<sub>3</sub> (6.00 g, 72.0 mmol) in THF–H<sub>2</sub>O (1:1, 24 mL) was added Boc<sub>2</sub>O (5.76 g, 26.4 mmol). After being stirred for 8 h at rt, the reaction mixture was quenched with 1N HCl and extracted with CHCl<sub>3</sub>. The combined organic solution was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. To a stirred solution of the crude mixture, **20** (4.45 g, 12.14 mmol), NaHCO<sub>3</sub> (5.10 g, 60.7 mmol) and Glyceroacetone-Oxyma (5.54 g, 24.3 mmol) in DMF–H<sub>2</sub>O (9:1, 60 mL), was added EDCI (4.65 g, 24.3 mmol). After being stirred for 13 h at rt, the reaction mixture was quenched with H<sub>2</sub>O and extracted with EtOAc. The combined organic solution was washed with 1N HCl (aq.), saturated NaHCO<sub>3</sub> (aq.), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 33:67 to 20:80) to afford **21** (5.35 g, 9.71 mmol, 80%) [4]: TLC (hexanes/EtOAc 20:80)  $R_f$  = 0.30; IR (thin film)  $\nu_{\max}$  = 3303 (br), 2931, 1692, 1637, 1613, 1542, 1504, 1465, 1366, 1264, 1238, 1219, 1193, 1159, 1120, 1111, 1036, 918, 841, 827, 772 cm<sup>–1</sup>; <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  7.19 (dd,  $J$  = 8.4, 4.7 Hz, 2H), 7.14 (d,  $J$  = 8.8 Hz, 2H), 6.91 (dd,  $J$  = 9.1, 1.1 Hz, 4H), 6.22 (brs, 1H), 4.77 (brs, 1H), 4.72 (brs, 1H), 4.44 (tt,  $J$  = 7.2,

3.5 Hz, 1H), 4.35 (d,  $J$  = 5.6 Hz, 1H), 4.29 (d,  $J$  = 5.5 Hz, 1H), 3.48 (ddt,  $J$  = 11.6, 7.6, 3.8 Hz, 2H), 3.20–3.05 (m, 4H), 2.22 (t,  $J$  = 7.1 Hz, 2H), 2.18–2.07 (m, 2H), 1.98–1.89 (m, 2H), 1.81 (quin,  $J$  = 6.9 Hz, 2H), 1.43 (s, 9H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  172.31, 157.99, 156.40, 155.72, 155.70, 142.76, 128.90 (2C), 128.56 (2C), 122.52 (3C), 116.76 (3C), 79.32, 72.55, 46.87, 44.10, 43.12, 39.76, 33.69, 30.15, 28.38 (3C), 26.35; HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{28}\text{H}_{37}\text{F}_3\text{N}_3\text{O}_5$   $[\text{M}+\text{H}]^+$  552.2685, found: 552.2701.



**4-Amino-N-(4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)butanamide (8):** To a stirred solution of **22** (3.81 g, 6.99 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was added TFA (5 mL). The reaction mixture was stirred for 3 h at rt, and all volatile were evaporated *in vacuo*. The residue was neutralized with aq.  $\text{NaHCO}_3$  extracted with  $\text{CHCl}_3$ . The combined organic extracts were dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The crude mixture of **8** was used for next reaction without purification.



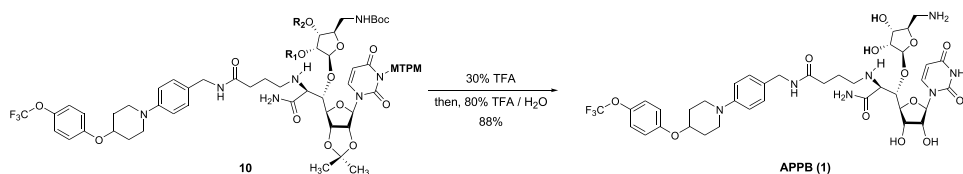
**((2R,3R,4R,5S)-2-(((tert-Butoxycarbonyl)amino)methyl)-5-(((1S)-1-((3aR,4R,6R,6aR)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-2,3-dihydroxy-5-phenylpentyl)oxy)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((trisisopropylsilyl)oxy)pentanoate) (23):** To a stirred solution of **6** (4.71 g, 2.99 mmol) and lepidine (2.37 mL, 17.9 mmol) in  $t\text{-BuOH}/\text{THF}/\text{H}_2\text{O}$  (1:1:1, 180 mL) were added  $\text{K}_2\text{CO}_3$  (2.06 g, 14.9 mmol),  $\text{K}_3\text{Fe}(\text{CN})_6$  (4.91 g, 14.9 mmol) and  $\text{K}_2\text{OsO}_4 \cdot 2\text{H}_2\text{O}$  (1.10 g, 2.99 mmol) at rt. After being stirred for 12 h, the reaction mixture were added  $\text{K}_2\text{CO}_3$  (2.06 g, 14.9 mmol),  $\text{K}_3\text{Fe}(\text{CN})_6$  (4.91 g, 14.9 mmol) and  $\text{K}_2\text{OsO}_4 \cdot 2\text{H}_2\text{O}$  (1.10 g, 2.99 mmol). After 20 h, the reaction mixture was diluted with EtOAc and quenched with saturated aq.  $\text{Na}_2\text{SO}_3$ . The heterogeneous mixture was stirred for 30 min, and extracted with EtOAc. The combined organic solution was washed with 1N HCl, saturated aq.  $\text{NaHCO}_3$ , dried over  $\text{Na}_2\text{SO}_4$ , and concentrated *in vacuo*. The crude mixture was passed through a silica gel pad (hexanes/EtOAc 75:25 to 50:50) to afford **23** (3.76 g, 2.33 mmol, 78%) as diastereomeric mixture. This mixture was used for next reaction without further purification. Data for less-polar diastereomer: TLC (hexanes/EtOAc 67:33)  $R_f$  = 0.30;  $[\alpha]_D^{22}$  0.210 ( $c$  = 1.62,  $\text{CHCl}_3$ ); IR (thin film)  $\nu_{\text{max}}$  = 3444 (br), 2941, 2866, 1741, 1719, 1675, 1600, 1556, 1457, 1382, 1367, 1278, 1249, 1216, 1160, 1098, 1070, 1049, 1013, 998, 882, 867, 754, 681  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.52 (dd,  $J$  = 8.4, 3.6 Hz, 1H), 7.31 (d,  $J$  = 2.0 Hz, 2H), 7.30–7.27 (m, 2H), 7.25–7.14 (m, 6H), 6.85 (d,  $J$  = 3.4 Hz, 2H), 6.50 (d,  $J$  = 5.9 Hz, 1H), 5.75 (dd,  $J$  = 17.6, 8.0 Hz, 1H), 5.63 (d,  $J$  = 22.1 Hz, 1H), 5.58–5.52 (m, 2H), 5.48 (d,  $J$  = 9.7 Hz, 1H), 5.21 (q,  $J$  = 7.3, 6.2 Hz, 2H), 5.11 (d,  $J$  = 6.8 Hz, 1H), 5.01 (dd,  $J$  = 8.4, 4.7 Hz, 1H), 4.85–4.78 (m, 2H), 4.25 (d,  $J$  = 5.6 Hz, 1H), 4.16 (dt,  $J$  = 8.6, 4.4 Hz, 1H), 4.03 (dd,  $J$  = 14.2, 5.1 Hz, 1H), 3.90 (d,  $J$  = 1.8 Hz, 1H), 3.78 (d,  $J$  = 1.8 Hz, 4H), 3.77–3.71 (m, 4H), 3.69–3.62 (m, 2H), 3.39–3.22 (m, 2H), 2.97–2.86 (m, 2H), 2.77–2.66 (m, 2H), 2.34–2.18 (m, 5H), 2.12–2.00 (m, 1H), 1.91–1.67 (m, 2H), 1.64–1.51 (m, 4H), 1.42 (s, 6H), 1.35 (d,  $J$  = 3.9 Hz, 3H), 1.13–0.99 (m, 41H), 0.99–0.94

(m, 6H), 0.86 (dtd,  $J = 9.1, 6.6, 2.2$  Hz, 3H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  170.85, 170.74, 170.70, 170.70, 162.10, 162.09, 159.44, 159.44, 156.05, 150.59, 150.53, 141.92, 141.89, 136.87, 136.84, 135.25, 135.09, 133.96, 133.77, 131.21, 131.17, 129.37, 129.32, 128.43 (2C), 128.38 (2C), 126.22, 126.14, 125.81, 125.36, 125.26, 115.27, 114.97, 80.36, 80.34, 79.85, 79.67, 79.58, 74.64, 74.62, 74.60, 73.82, 73.77, 73.72, 70.31, 70.31, 59.94, 59.90, 55.69, 46.13, 45.92, 44.72, 34.63, 34.50, 32.58, 32.57, 32.55, 32.54, 31.76, 29.69, 29.03, 28.35, 27.28, 27.22, 26.88, 25.32, 25.25, 20.68, 18.04 (1C), 11.88 (3C), 11.86 (3C), 11.43; HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{79}\text{H}_{120}\text{Cl}_4\text{N}_3\text{O}_{19}\text{Si}_2$  [M+H] 1610.6809, found: 1610.6827. Data for polar diastereomer: TLC (hexanes/EtOAc 67:33)  $R_f = 0.20$ ;  $[\alpha]_D^{22} 0.071$  ( $c = 1.08$ ,  $\text{CHCl}_3$ ); IR (thin film)  $\nu_{\text{max}} = 3413$  (br), 2941, 2866, 1719, 1675, 1457, 1367, 1278, 1248, 1219, 1160, 1099, 1070, 1049, 882, 772  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.58 (d,  $J = 8.5$  Hz, 1H), 7.31–7.29 (m, 2H), 7.28 (s, 2H), 7.24–7.11 (m, 6H), 6.84 (d,  $J = 1.4$  Hz, 2H), 6.51 (d,  $J = 6.6$  Hz, 1H), 5.90 (dd,  $J = 6.3, 2.7$  Hz, 1H), 5.84 (t,  $J = 8.2$  Hz, 1H), 5.61–5.41 (m, 2H), 5.23–5.10 (m, 2H), 5.04 (t,  $J = 5.8$  Hz, 2H), 4.86–4.77 (m, 1H), 4.68 (ddd,  $J = 21.0, 6.3, 2.8$  Hz, 1H), 4.57 (dt,  $J = 10.8, 3.8$  Hz, 1H), 4.25–4.14 (m, 1H), 4.06–3.98 (m, 1H), 3.92–3.84 (m, 1H), 3.80–3.71 (m, 6H), 3.47–3.23 (m, 2H), 2.92–2.83 (m, 2H), 2.77–2.66 (m, 2H), 2.31–2.20 (m, 4H), 2.19–2.06 (m, 2H), 1.92–1.66 (m, 3H), 1.63–1.53 (m, 6H), 1.42 (d,  $J = 3.7$  Hz, 2H), 1.36 (s, 6H), 1.10–0.94 (m, 50H), 0.91–0.81 (m, 3H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  171.04, 171.01, 170.93, 170.92, 162.00, 159.38, 150.79, 136.92, 136.91, 135.45, 131.30, 131.28, 129.29, 129.28, 128.46 (2C), 128.42 (2C), 126.09, 125.95, 125.93, 115.24, 81.03, 81.01, 79.95, 79.67, 75.03, 75.00, 74.98, 72.17, 70.38, 70.31, 69.52, 69.49, 59.95, 59.91, 55.69, 55.67, 46.13, 45.93, 44.86, 44.66, 35.27, 35.25, 34.64, 32.63, 32.59, 32.58, 31.95, 28.32, 27.38, 27.37, 27.36, 27.28, 27.27, 27.20, 26.89, 25.26, 18.05 (12C), 11.88 (3C), 11.87 (3C); HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{79}\text{H}_{120}\text{Cl}_4\text{N}_3\text{O}_{19}\text{Si}_2$  [M+H] 1610.6809, found: 1610.6831.

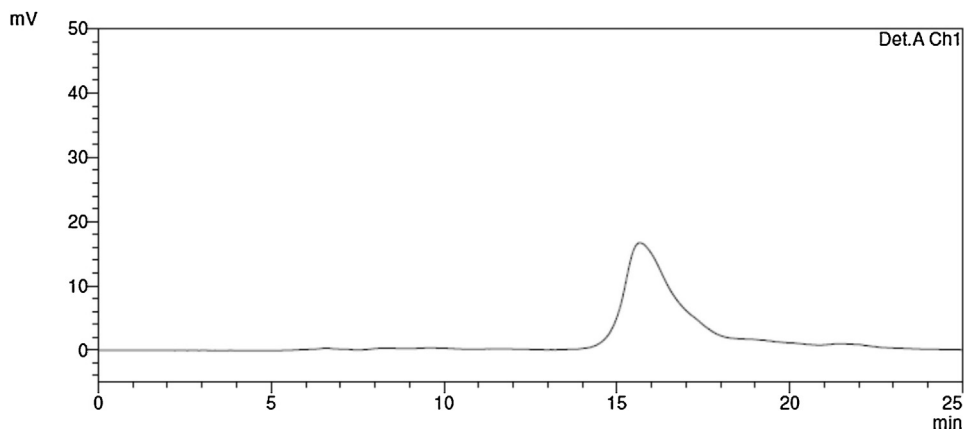
**(2R,3R,4R,5S)-2-(((tert-Butoxycarbonyl)amino)methyl)-5-(((1S,2R)-2-cyano-1-((3aR,4R,6-R,6aR)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-2-((4-oxo-4-((4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)amino)butyl)amino)ethoxy)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyl)oxy)pentanoate) (9):** To a stirred suspension of **23** (3.76 g, 2.33 mmol) and  $\text{NaHCO}_3$  (0.98 g, 11.6 mmol) in  $\text{CH}_2\text{Cl}_2$  (46.6 mL) was added  $\text{Pb}(\text{OAc})_4$  (2.06 g, 4.66 mmol) at  $0^\circ\text{C}$ . The reaction mixture was stirred for 2 h at  $0^\circ\text{C}$  and quenched with saturated aq.  $\text{NaHCO}_3$ , and extracted with EtOAc. The combined organic extracts were dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The crude mixture of aldehyde **7** was used for the next reaction without purification. To a stirred solution of **7** (3.44 g, 2.33 mmol) and **8** (3.15 g, 6.99 mmol) in  $\text{CH}_2\text{Cl}_2$  (30 mL) was added MS3A (7.5 g) followed by  $\text{Ti}(\text{OiPr})_4$  (6.89 mL, 23.3 mmol). After 6 h, the reaction was added TMSCN (2.91 mL, 23.3 mmol) and stirred for 12 h at rt. After completion, the reaction mixture was quenched with saturated aq.  $\text{NaHCO}_3$ , and extracted with EtOAc. The combined organic extracts were dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (hexanes/EtOAc 80:20 to 60:40) to afford **9S** (3.15 g, 1.63 mmol, 70% for 2 steps); TLC (hexanes/EtOAc 50:50)  $R_f = 0.40$ ;  $[\alpha]_D^{21} +0.102$  ( $c = 0.75$ ,  $\text{CHCl}_3$ ); IR (thin film)  $\nu_{\text{max}} = 3342$  (br), 2941, 2866, 1718, 1675, 1505, 1464, 1243, 1164, 1101, 1071, 883, 772, 688  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.49 (dd,  $J = 8.5, 4.3$  Hz, 1H), 7.32 (d,  $J = 2.0$  Hz, 1H), 7.22–7.11 (m, 7H), 6.94–6.88 (m, 5H), 6.86 (d,  $J = 6.5$  Hz, 2H), 6.50 (d,  $J = 8.6$  Hz, 1H), 6.25–6.16 (m, 1H), 5.73 (dd,  $J = 22.2, 8.0$  Hz, 1H), 5.60 (t,  $J = 8.8$  Hz, 1H), 5.56–5.41 (m, 3H), 5.21 (d,  $J = 4.4$  Hz, 1H), 5.05–4.98 (m, 2H), 4.94–4.77 (m, 2H), 4.53–4.37 (m, 3H), 4.25–4.16 (m, 2H), 4.05–3.98 (m, 1H), 3.80–3.69 (m, 6H), 3.68–3.63 (m, 1H), 3.56 (dd,  $J = 17.3, 3.4$  Hz, 1H), 3.48 (ddt,  $J = 11.6, 7.2, 4.0$  Hz, 2H), 3.44–3.29 (m, 1H), 3.08 (dq,  $J = 9.5, 5.3, 4.8$  Hz, 2H), 2.95 (dt,  $J = 11.4, 5.5$  Hz, 1H), 2.47 (td,  $J = 12.0, 11.4, 5.7$  Hz, 1H), 2.36–2.14 (m, 5H), 2.13–2.05 (m, 2H), 1.97–1.85 (m, 3H), 1.84–1.75 (m, 1H), 1.58 (t,  $J = 6.9$  Hz, 2H), 1.55–1.50 (m, 4H), 1.40 (s, 9H), 1.33 (d,  $J = 4.8$  Hz, 3H), 1.28–1.23 (m, 3H), 1.08–1.02 (m, 42H), 1.01 (s, 6H), 0.94 (d,  $J = 2.1$  Hz, 6H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  172.4, 171.0, 170.9, 159.5, 155.8, 150.9, 150.7, 142.8, 136.9, 136.8, 135.3, 135.1, 134.13, 134.05, 133.86, 133.85, 133.78, 131.2, 131.1, 129.42, 129.37, 129.0, 126.4, 126.2, 125.5, 125.2, 122.5 (2C), 121.8, 119.3, 118.4, 116.8 (2C), 116.6 (2C), 115.4, 115.3, 114.71, 114.66, 106.4, 102.3, 102.2, 84.8, 80.7, 80.6, 79.9, 79.8, 79.3, 76.2, 74.32, 74.30, 72.9, 60.38, 60.35, 60.0, 59.9, 55.72, 55.71, 52.0, 46.6, 46.2, 45.9, 44.84, 44.77, 42.99, 42.96, 42.4, 41.2, 33.53, 33.49, 32.6, 32.5, 30.3, 28.4, 27.3 (2C), 27.17, 27.16, 27.1, 25.4, 18.1 (12C), 14.2, 14.1, 11.91 (3C), 11.90 (3C); HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{94}\text{H}_{135}\text{Cl}_4\text{F}_3\text{N}_7\text{O}_{26}\text{Si}_2$  [M+H] 1934.8007, found: 1934.8021.



**(2S,3R,4R,5R)-2-((1S,2S)-3-Amino-1-((3aR,4R,6R,6aR)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyl-tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-3-oxo-2-((4-oxo-4-((4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)amino)butyl)amino)propoxy)-5-(((tert-butoxycarbonyl)amino)methyl)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyl)oxy)pentanoate) (10):** To a stirred solution of **9S** (3.15 g, 1.63 mmol) in EtOH/H<sub>2</sub>O (9:1, 10 mL) were added HgCl<sub>2</sub> (0.89 g, 3.26 mmol) and acetaldoxime (0.99 mL, 16.3 mmol) at rt. After being stirred for 10 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was quenched with saturated aq. NaHCO<sub>3</sub>, extracted with CHCl<sub>3</sub>. The combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (CHCl<sub>3</sub>/MeOH 99.5:0.5–99.2:0.8–98.8:1.2) to afford **10** (2.64 g, 1.35 mmol, 83%); TLC (CHCl<sub>3</sub>/MeOH 95:5) *R<sub>f</sub>* = 0.30; [ $\alpha$ ]<sub>D</sub><sup>21</sup> +0.144 (*c* = 0.53, CHCl<sub>3</sub>); IR (thin film)  $\nu_{\text{max}}$  = 3335 (br), 2940, 2866, 1719, 1676, 1505, 1464, 1367, 1242, 1162, 1101, 1070, 882, 681 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.53 (dd, *J* = 8.6, 5.1 Hz, 1H), 7.30 (s, 1H), 7.28–7.22 (m, 2H), 7.21–7.12 (m, 6H), 6.91 (d, *J* = 8.5 Hz, 4H), 6.86 (d, *J* = 2.6 Hz, 2H), 6.51 (d, *J* = 8.7 Hz, 1H), 5.94 (brs, 1H), 5.79–5.67 (m, 3H), 5.56–5.47 (m, 2H), 5.17 (brs, 1H), 5.06 (s, 1H), 4.96 (brs, 1H), 4.82–4.73 (m, 2H), 4.43 (tt, *J* = 7.8, 3.8 Hz, 1H), 4.39–4.28 (m, 3H), 4.21 (brs, 1H), 4.13 (brs, 1H), 3.78 (s, 3H), 3.73 (q, *J* = 7.4 Hz, 5H), 3.67 (brs, 1H), 3.48 (ddd, *J* = 11.7, 7.2, 3.7 Hz, 2H), 3.41–3.28 (m, 1H), 3.17 (s, 1H), 3.09 (ddd, *J* = 12.2, 8.2, 3.3 Hz, 2H), 2.80–2.60 (m, 2H), 2.38–2.15 (m, 7H), 2.13–2.05 (m, 2H), 1.93 (ddd, *J* = 12.8, 8.0, 3.7 Hz, 2H), 1.85–1.79 (m, 2H), 1.54 (s, 3H), 1.42 (s, 9H), 1.34 (s, 3H), 1.04 (d, *J* = 2.8 Hz, 42H), 1.01 (s, 6H), 0.96 (s, 6H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  162.1, 162.0, 159.6, 159.5, 156.2, 155.8, 150.9, 150.4, 142.80, 142.78, 136.88, 136.86, 135.23, 135.21, 133.9, 133.6, 131.33, 131.30, 131.29, 129.40, 129.37, 129.2, 129.1, 129.02, 128.98, 126.24, 126.22, 126.21, 125.40, 125.36, 124.5, 124.4, 123.20, 123.19, 122.5 (2C), 121.8, 120.1, 119.3, 116.8 (2C), 115.4, 80.4, 80.02, 79.99, 79.96, 79.95, 79.92, 79.87, 79.85, 79.83, 74.51, 74.50, 72.7, 70.4, 70.3, 69.5, 60.0, 59.9, 55.73, 55.72, 46.7, 46.19, 46.15, 46.13, 46.11, 46.10, 46.07, 46.0, 44.8, 34.7, 34.5, 32.61, 32.58, 30.2, 29.7, 29.64, 29.60, 28.50, 28.45, 28.42, 28.38, 28.34, 27.25 (2C), 27.19, 27.16, 25.31, 25.29, 25.27, 18.1 (12C), 14.1, 12.2, 11.9 (6C); HRMS (ESI+) *m/z* calcd for C<sub>94</sub>H<sub>137</sub>Cl<sub>4</sub>F<sub>3</sub>N<sub>7</sub>O<sub>21</sub>Si<sub>2</sub> [M+H] 1952.8112, found: 1952.8098.



**4-(((2S,3S)-1-Amino-3-(((2S,3R,4S,5R)-5-(aminomethyl)-3,4-dihydroxytetrahydrofuran-2-yl)oxy)-3-((2S,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-1-oxopropan-2-yl)amino)-N-(4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)butanamide (1):** To a stirred solution of **10** (2.64 g, 1.35 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was added TFA (10 mL). The reaction mixture was stirred for 3 h at rt, and all volatile were evaporated *in vacuo*. To a stirred solution of the crude mixture in H<sub>2</sub>O (5 mL) was added TFA (20 mL). The reaction mixture was stirred for 2 days at rt, and all volatile were evaporated *in vacuo*. The crude mixture was purified by DOWEX (50W x 4) ion exchange resin. The resin was washed with MeOH/H<sub>2</sub>O (4:1) and MeOH. The crude product (TFA salt) was dissolved in MeOH (10 mL) and absorbed on DOWEX (50W x 4); the crude **1** was not detected by TLC (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O/50% aqueous ammonia 56:42:7:3). The resins were washed with MeOH and eluted with MeOH/50% aqueous ammonia (10:1). The eluate was concentrated under reduced pressure and the resultant aqueous solution was lyophilized. The resulted mixture was purified by C18 reverse-phase HPLC [column: HYPERSIL GOLD™ (175 Å, 12 μm,



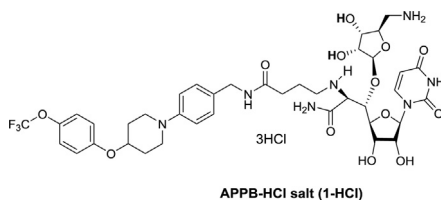
**Fig. 1.** HPLC analysis of **1**.

Area % purity: 96.8%.

Conditions: column: Phenomenex Kinetex 5  $\mu$ m XB-C18 100 Å 250  $\times$  4.60 mm column, solvents: 85:15 MeOH:0.05M  $\text{NH}_4\text{HCO}_3$  in water, UV: 254 nm, flow rate: 0.5 mL/min.

150  $\times$  20 mm), solvents: 80:20 MeOH:0.05M  $\text{NH}_4\text{HCO}_3$  in  $\text{H}_2\text{O}$ , flow rate: 6.0 mL/min, UV: 254 nm, retention time: 14 min] to afford **1** (1.05 g, 1.19 mmol, 88%); TLC (*n*-butanol/ethanol/ $\text{CHCl}_3$ /28% aqueous ammonia 4:7:2:7)  $R_f$  = 0.50;  $[\alpha]^{21}_D$  +0.375 ( $c$  = 0.30, methanol); IR (thin film)  $\nu_{\text{max}}$  = 3352 (br), 2932, 1677, 1505, 1243, 1201, 1136, 801, 722  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.78 (d,  $J$  = 8.1 Hz, 1H), 7.18 (dd,  $J$  = 9.0, 3.5 Hz, 4H), 7.00 (dd,  $J$  = 16.0, 8.6 Hz, 4H), 5.77 (d,  $J$  = 2.9 Hz, 1H), 5.73 (d,  $J$  = 8.1 Hz, 1H), 5.14 (s, 1H), 4.57–4.50 (m, 1H), 4.28 (s, 2H), 4.22–4.13 (m, 3H), 4.10 (dd,  $J$  = 8.6, 4.4 Hz, 1H), 4.07–3.98 (m, 2H), 3.52–3.46 (m, 3H), 3.44 (d,  $J$  = 8.8 Hz, 1H), 3.17 (d,  $J$  = 13.0 Hz, 1H), 3.14–3.02 (m, 3H), 2.60 (ddq,  $J$  = 18.4, 11.8, 6.9 Hz, 2H), 2.29 (td,  $J$  = 7.3, 2.8 Hz, 2H), 2.12 (dd,  $J$  = 14.5, 5.6 Hz, 2H), 1.93–1.73 (m, 4H), 1.39–1.25 (m, 2H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  175.6, 166.2, 157.6, 152.0, 142.6, 131.2, 129.6 (2C), 123.6 (2C), 118.11 (2C), 118.07 (2C), 110.5, 102.7, 92.3, 85.3, 81.4, 80.4, 76.5, 75.1, 74.1 (2C), 73.0, 71.3, 64.4, 43.7, 43.6, 34.7, 31.5, 26.9; HRMS (ESI+)  $m/z$  calcd for  $\text{C}_{39}\text{H}_{51}\text{F}_3\text{N}_7\text{O}_{13}$   $[\text{M}+\text{H}]$  882.3497, found: 882.3512 (Fig. 1).

#### Preparation of HCl salt of **1**



To a stirred solution of **1** (1.05 g, 1.19 mmol) in MeOH (50 mL) was added ice cold 1N HCl (23.8 mL, 23.8 mmol) dropwise. After being stirred for 1 h at rt, the solution was concentrated under reduced pressure and the resultant aqueous solution was lyophilized to give **1**•HCl salt (Fig. 2).

#### Determination of solubility of **1**•HCl in 0.9% NaCl (saline)

A suspension of **1**•HCl (4.0 mg) in 0.9% NaCl (30  $\mu$ L) was stirred for 24 h, and the precipitate was separated by centrifugation at  $10,000 \times g$  for 5 min. The upper solution (1  $\mu$ L) was analyzed via C18 reverse-phase HPLC [column: Kinetex (100 Å, 5  $\mu$ m, 250  $\times$  4.60 mm), solvents: 70:30 MeOH:

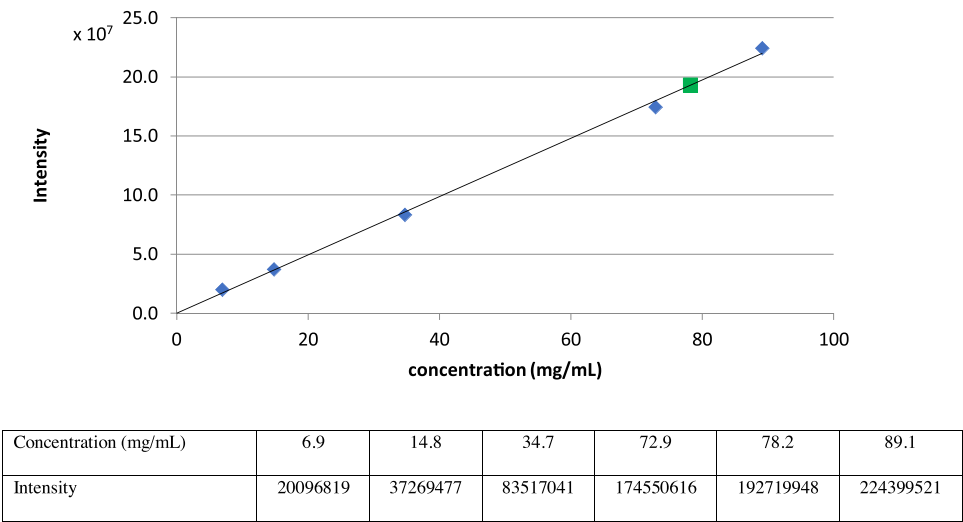


Fig. 2. Water solubility of **1•HCl** in saline.

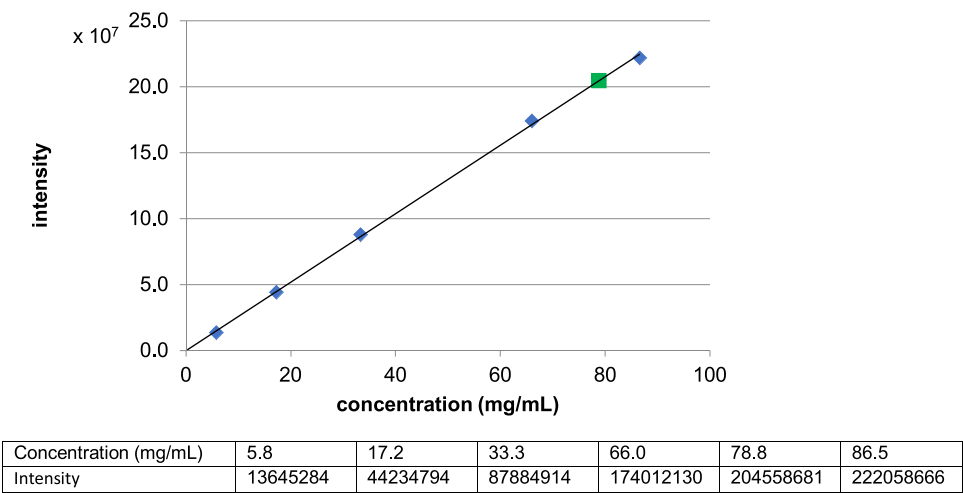
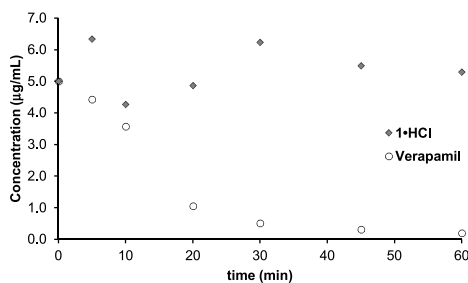


Fig. 3. Water solubility of **1•HCl** in PBS (pH7.4).

0.05 M  $\text{NH}_4\text{HCO}_3$  aq., flow rate: 0.5 mL/min, UV: 254 nm, retention time: 12.0 min]. The area of the peak for **1** was quantified. The concentrations were determined via the HPLC intensity-concentration curves [7–9].

*Determination of solubility of **1•HCl** in PBS (pH7.4) buffer*

A suspension of **1•HCl** (3.8 mg) in phosphate buffered saline (pH 7.4, 30  $\mu\text{L}$ ) was stirred for 24 h, and the precipitate was separated by centrifugation at  $10,000 \times g$  for 5 min. The upper solution (1  $\mu\text{L}$ ) was analyzed via C18 reverse-phase HPLC [column: Kinetex (100 Å, 5  $\mu\text{m}$ ,  $250 \times 4.60$  mm), solvents: 70:30 MeOH:0.05 M  $\text{NH}_4\text{HCO}_3$  aq., flow rate: 0.5 mL/min, UV: 254 nm, retention time: 12.0 min]. The area of the peak for **1** was quantified. The concentrations were determined via the HPLC intensity-concentration curves [7–9] (Fig. 3).



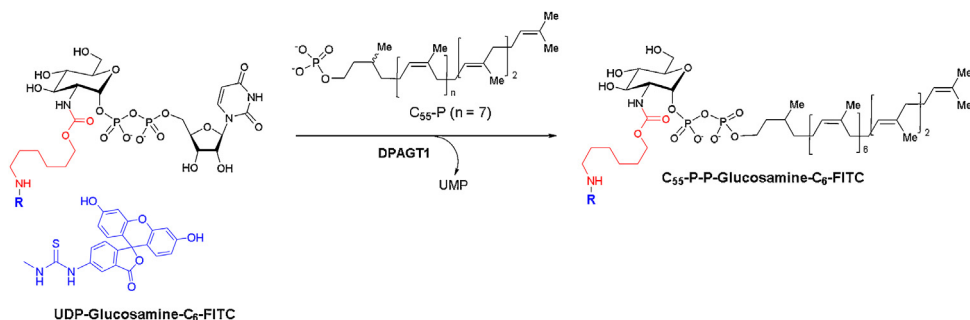
### Microsomal stability

Pooled Sprague-Dawley rat liver microsomes were purchased from Corning Life Sciences (Oneonta, NY, USA). Microsomes (20 mg/mL) were thawed on ice and diluted with PBS, potassium phosphate buffer (100 mM, pH: 7.4) at a 1:8 ratio in 1.5 mL Eppendorf tubes. Stock solutions of **1•HCl** and verapamil (positive control) were made by diluting 10 mg/mL solutions. From the drug stock solution, 10  $\mu$ L was diluted with 390  $\mu$ L of buffer (0.1 mg/400  $\mu$ L). The diluted microsomes (390  $\mu$ L) were reacted with 10  $\mu$ L of the diluted drug solution and allowed to equilibrate for 5 min while shaking at 440 rpm. NADPH (10 mg/200  $\mu$ L; 1000 $\times$  drug concentration) was used as a co-factor for this reaction, and 100  $\mu$ L was added to the solution after equilibration. Ice cold methanol (200  $\mu$ L) was used to quench the reaction mixture (50  $\mu$ L aliquots) at 0, 5, 10, 20, 30, 45 and 60 min. The samples containing methanol was lyophilized to remove all volatiles. The residue was dissolved in 1N HCl aq. (10  $\mu$ L) and MeOH (40  $\mu$ L). The resulting solution (20  $\mu$ L) was injected to LC-MS. MS solvent 90:10 acetonitrile/0.05% formic acid in water. Flow rate: 0.5 mL/min (Fig. 4).

### DPAGT1 assay

The enzymatic substrate, UDP-Glucosamine-C<sub>6</sub>-FITC was chemically synthesized according to the reported procedures [10]. DPAGT1 was expressed in suspended Expi293 cells for 36 h. The cells were lysed by drawing through a 26 g needle (10 times) and membrane protein was extracted using buffer containing 1% DM (decyl β-D-maltopyranoside) detergent. DPAGT1 was purified using HA (hemagglutinin)-agarose resin and a superdex 200 size exclusion column (Fig. 5).

UDP-Glucosamine-C<sub>6</sub>-FITC (2 mM stock solution, 0.56  $\mu$ L), MgCl<sub>2</sub> (0.5 M, 4  $\mu$ L),  $\beta$ -mercaptoethanol (50 mM, 5  $\mu$ L), CHAPS (20%, 2.5  $\mu$ L), Tris buffer (pH 8.0, 50 mM), C<sub>55</sub>-dolichyl phosphate (4 mM, 1.68  $\mu$ L), and **1•HCl** (0–50  $\mu$ g/mL in Tris buffer) were placed in a 500  $\mu$ L Eppendorf tube. To a stirred



**Fig. 5.** DPAGT1-catalyzed reactions.

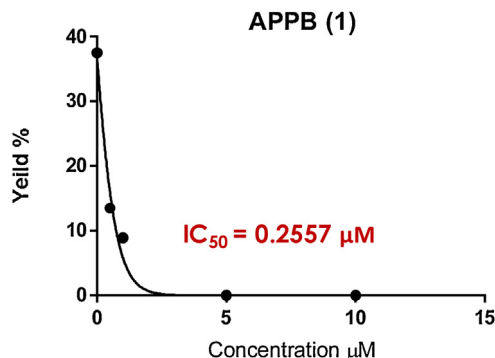


Fig. 6.  $IC_{50}$  curve for APPB (1).

reaction mixture, DPACT1 solution (10  $\mu$ L) was added (total volume of reaction mixture: 50  $\mu$ L adjust with Tris buffer). The reaction mixture was incubated for 4 h at 37 °C and quenched with *n*-butanol (150  $\mu$ L). Two phases were mixed *via* vortex and centrifuged at  $10,000 \times g$  for 3 min. The upper organic phase was assayed *via* reverse-phase HPLC. The organic phase (30  $\mu$ L) was injected into HPLC (solvent: gradient elution of 85:15–95:5 MeOH/0.05 M aq.  $NH_4HCO_3$  over 20 min; UV: 485 nm; flow rate: 0.5 mL/min; column: Kinetex 5  $\mu$ m C8, 100 Å,  $150 \times 4.60$  mm), and the area of the peak for C<sub>55</sub>-P-P-glucosamine-C<sub>6</sub>-FITC was quantified to obtain the  $IC_{50}$  value. The  $IC_{50}$  values were calculated from plots of the percentage product inhibition versus the inhibitor concentration (Fig. 6).

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.mex.2019.09.031>.

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